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NUCLEAR WEAPONS EFFECTS RESEARCH PROGRESS REPORT (TERMINAL).

TITLE: "Early Effects of Renal X-Irradiation".

CONTRACT NO. DA-49-193-2089

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OBJECTIVE(S).

I. Primary. Definition of changes in specific renal functions during the early days and weeks after exposure to heavy X-irradiation.

II. Secondary. Definition of significance, rationalization and numerical expression of the radioisotope renogram as a quantitative index of overall function of separate kidneys. (This study was undertaken because of availability of apparatus and techniques and of necessarily time lapses between experiments directed toward primary aim. It was not the basis of the contract request. Studies in this connection were supported primarily by grants made by the Youngstown (Ohio) Heart Association, the Cleveland Foundation and the National Institutes of Health, National Heart Institute (H-4980). Thus it involved charges against the contract only for part-time of professional and technical personell. The major part of this project was substantially completed some months prior to completion of studies on the primary objective and a paper thereon is in press (J. Lab. Clin. Med., 1963). Results are reported since the technique would seem advantageous in screening, detecting and evaluating renal disease in military as in other subjects.

I. PRIMARY OBJECTIVE

BACKGROUND AND COURSE OF STUDY.

1. Primary Objective. The histologic changes of "irradiation nephritis", which appear some weeks or months after heavy renal irradiation and experimentally and clinically, are frequently associated with arterial hypertension have been widely described. Recent reports dealing with the experimental syndrome include that of Wilson (Lancet i:9, 1958) and with the clinical, that of Luxton (Lancet ii:121, 1961).

Since it seemed desirable to determine possible early effects on specific renal functions of such irradiation, i.e., functional changes that might precede gross histologic changes, studies were initiated by Klapproth, Takagi and Corcoran. A preliminary report on effects in dogs with single, subcutaneously explanted kidneys was published in Lancet, ii, 161, 1959). These studies indicated depression of specific functions of PAH and glucose transport and, at 3-4 weeks, of glomerular filtration: the data suggested a dissociation between relatively early depression of maximum capacity for PAH transport (TmPAH) and capacity for glucose reabsorption (TmG). This formed the basis for application for a contract that would enable us to extend the observations.

Since that time, Asscher and Anson (Lancet i:1109, 1960) showed that irradiation (1200 r) of rat kidney was followed in a few days by diffuse loss of alkaline phosphatase, some of which appeared in urine, but recovery thereof before the onset of fibrosis. Further, Rich, Larsen and Spargo (Arch. Path. 72:388, 1961) using heavier doses of x-ray, concluded that irradiation modifies processes of H^+ transfer, oxidative phosphorylation, dehydrogenase, diaphorase and phosphatase, at about 3 -7 weeks and that these changes "tended to parallel the parenchymal loss and progressive renal fibrosis which characterized the post-irradiation " this implies that cytochemical changes were relatively late in appearance.

Also since these studies began, Wilson (Proc. Prague Symposium on the Pathogenesis of Essential Hypertension, p. 413 seq.) and Asscher, Wilson and Anson (Lancet i:580, 1961) have described experiments in rats that show that irradiated mesenteric arteries, while demonstrating no apparent histologic change, are abnormally susceptible to necrosis of the wall consequent on even mild renal hypertension. More recently, Asscher and Anson (Lancet ii:1343, 1962) have shown that the same applied to cerebro-spinal arteries of rats i.e., that they are abnormally susceptible to severe hypertensive vascular disease as a sequel to mild renal hypertension. Since no structural change could be shown in the affected vessel walls prior to onset of hypertension, it appeared either that the muscle was rendered abnormally responsive to damaging effects of increases in lateral arterial pressure, as by stretching or constriction at the site, or possibly, abnormally sensitive to vasoconstrictor effects of circulating pressor agents that may act in renal hypertension.

(a) Initial Studies. These began as extension of the procedures and observations made by Klapproth et al. (l.c. above), i.e., measurements of CPAH (at low plasma concentrations) CCr, TmPAH and TmG, at intervals before and after irradiation of a single explanted kidney in a previously uninephrectomized dog, under conditions of hydropenia (12 hours fluid deprivation) osmotic diuresis (infusion fluid mannigol content ca. 10-15 per cent), and light pentobarbital anesthesia.

The experiments were begun in association with Dr. Klapproth. After control levels of function were established by weekly tests at intervals of about 3 weeks after contralateral nephrectomy, irradiation was given in 2 or 3 doses of 600-800 r. each and at intervals of 3 days, using a 250 KV apparatus, Thorius filter No 2, HVL 3.2 mm. Cu.

In 3 animals in which control and experimental conditions seemed satisfactory, we were able to confirm the gradual onset of overall deterioration of functions (renal plasma flow, glomerular filtration, TmPAH and TmG) but were unable to confirm the existence of any dissociation between rates of change in the Tm's. However, at 1 - 3 weeks after irradiation, i.e., prior to gross defect in measured functions, notably glomerular filtration, we observed a large increment in the rate of urine flow during the tests. This, under the conditions of the experiment, indicated a defect in water and, possibly electrolyte reabsorption. However, at this time we were not fully equipped for electrolyte studies and had not obtained a Fiske Osmometer (later obtained through a grant from the Cleveland Foundation) and were unable to determine the nature or significance of the defect.

These experiments were terminated in Late July,1960 with resignation of Dr. Klapproth and the contract temporarily suspended, pending re-establishment of personnel and re-orientation of procedures. This began in Dec. 1961 with the acquisition to our staff of a Research Fellow, Dr. Akina Hirakawa of Kyoto, and of an Associate, Dr. Thomas Nolan, M.D. F.A.C.S. Orienting studies were begun. New plans were developed in consultation with Col. Joseph Goldstein, M.C., USA, who visited the laboratory March 2, 1961. These pointed toward a more fruitful and fundamental, as well as more critical approach to the problem. The contract was re-activated July 1, 1961 and its term later extended without additional funds, to Dec. 30th, 1962.

(b) Definitive Program. The basic aim remained unchanged. However, scope of study was extended to measurements of water and electrolyte (Na and K) transport, effects of drugs that alter these function (notably the so called salt-diuretics) and of responsiveness of renal vasculature to infusion of small amounts of angiotensin. As concerned water and electrolyte functions, it seemed likely that the kidney presents a unique situation for study among intact organs, in that it is possible to measure contents of its extracellular fluid, which should correspond to plasma, and of an extracellular fluid, in the urine, and thus perhaps define changes which may occur in irradiated tissues generally but which are inapparent because of the impossibility of separate analysis on fluids on two sides of the cell. Further, accuracy of observation was increased by planning simultaneous comparisons of the functions of a normal, untouched kidney with its irradiated, subcutaneously explanted twin, collecting urine separately from each. This last disposes of problems of fluctuations of renal function as the result of incidental factors, e.g. depth of anesthesia, extrarenal infection, changes in diet etc., which had several times previously disturbed the course of studies under way and toward which much effort had been expended.

An interim report (March 1, 1962) described early results and problems. The problems arose in part from the frequency of distemper in pound dogs- this was overcome by initial passive immunization of all animals - - and by difficulties with the "split-bladder" technique of Desautels (Surg. Gyn. Ob. 105:767, 1951) as modified (personal communication) by Drs. P. A. F Morris and Neil S. Bricker of St. Louis. Difficulties lay primarily in frequent obstruction of Teflon tubes sewn into each bladder pocket, with resultant infection of the kidney on the affected side. This effectively destroyed the course of an experiment as did, in some cases, the animals' tearing out the Teflon tubes. The technic was modified, primarily by making tubes larger and less subject to obstruction and also by daily irrigation of bladder segments with sterile saline and instillation of an antibiotic (usually neomycin).

A subsequent interim report (June 1, 1962) described further progres. Some disassociation of changes in TmPAH and TmG had been noted to follow irradiation. The changes, however, were not large and their significance was not apparent. The most obvious finding in a series of 6 dogs then studied or under study was establishment of a natriuretic, kaliuretic state, followed by a condition of impairment of ability for free water conservation (loss and even reversal of TmH_2O) with resistance to endogenous anti-diuretic hormones. Some evidence had been obtained of increased vascular responsiveness (vasoconstrictor) to infused angiotensin on the irradiated side.

An interim report to Nov. 1st, 1962, was furnished Nov. 17th, 1962, towards the conclusion of the experiments. These had been very vigorously prosecuted during the several preceeding months and the remainder of the year. This report again noted the decrements in renal ability to retain Na and K and, subsequently water, and suggests that these may arise from defect ir basic energy supply, possibly at the level of H^+ transfer, in distal and collecting and, probably proximal tubule cells. The report that follows brings these data together and extends them by noting observations on effects of salt-diuretics and angiotensin.

PROCEDURES.

Dogs were first passively immunized with distemper serum after selection for sex and size. They were then prepared (Dr. Nolan) by isolation of the bladder into 2 compartments, each drained through specially prepared Teflon tubes, sewn to bladder wall and skin. Each bladder compartment was rinsed daily with a saline-neomycin solution. The left kidney was subcutaneously explanted (Page and Corcoran i.e.).

Tests of kidney function were done after 12-15 hours fluid deprivation, under light pentobarbital sodium anesthesia. Infusion fluid contained 10 -15 per cent mannitol and sufficient concentrations of PAH and creatinine to maintain desired plasma concentrations. Infusions were delivered from a Harvard pump and fluid administered as 1 per cent NaCL to make up for urinary loss.

Each observation consisted of at least 3 periods of 10 -15 minutes each of urine collection, with blood sampling towards the mid-points of the 1st and 3rd periods. These data were averaged in collecting results. Typically, an experiment consisted of 3 periods (one observation) of control status, then 20-30 minute rest periods, during which infusion and re-infusion of fluid was maintained, following which an experimental observation (e.g. addition to infusion fluid of angiotensin) was made; this was followed by rest and renewed "control" observations before a new experimental procedure, (e.g. administration of a salt-diuretic) was begun. In most animals, blood pressures were determined at intervals, by femoral arterial puncture (Sanborn gauge and adaptation of 2 channel apparatus as manometer. Routine observations during control and experimental periods consisted usually of collection of data on CPAH (at low plasma concentrations), CCr, urine flow (V) COsm (osmolal clearance) and calculated, by difference of $Tm^{C}H_2O$, with associated measurements of urinary outputs (μEq/min.) of Na and K and, in most experiments, of serum Na. In 5 experiments among the series the measurements included observations of TmPAH (PAH in plasma at high concentrations) followed by a rest period during which infusion of hypertonic glucose was begun for measurement in 3 periods of urine collection of TmG. Six experiments included experimental periods during which angiotensin was infused (0.1-0.15 ug. angiotensin II amide per kg. per min.) and several, detailed below, observations on effects of other agents that influence reabsorption of electrolytes.

Tests were done at least once before and at regular intervals (3, 7, 14, 21 and 28 days) after X-irradiation. At the end of the period animal was

sacrificed and tissues from untouched and irradiated kidneys placed in fixatives and sent to Dr. F. K. Mostofi, Armed Forces Institute of Pathology, for histological study.

Doses of X-irradiation (administered at intervals as described above) varied from 2400 r (in most instances to 3000 r (Dog. GG).

RESULTS.

Significant changes were observed in CPAH, CCr, V, $Tm^{C}H_{2}O$, and in Na and K ouputs (UVNa and UV K). Changes in TmPAH tended to parallel changes in CPAH and those in TmG were in roughly constant proportion with CCr. Hence, while these data indicate preservation in relative integrity of complex proximal tubular transport systems, they are not here considered in detail.

The major changes are listed as percentile ratios of untouched (right) to the explanted, subsequently irradiated, left kidney, as observed during control periods (i.e., before administration of test agents). These data, with standard deviations and indications of significant differences (P greater than 0.01) are listed in Table I.

I. Effect of Operative Procedures. Equality of functions between the untouched and subcutaneously explanted kidneys established that operation as such did not influence kidney function.

II. Effect of Irraditation. (a) On "Basal" Functions.

(1) CPAH. This function, taken as roughly equivalent to renal plasma flow in the absence of severe deterioration of TmPAH (and presumption therefrom of a defect in extraction) was increased from the 3rd through the 14 post-irradiation day. It returned approximately to normal at the 21st day and was depressed below normal levels, possibly in part by loss of extraction, at the 28th day.

(2) CCr. The sequence of change in this function paralleled that of CPAH. Since CCr reflects merely the mechanical process of glomerular filtration, while CPAH reflects efficiency (in terms of extraction) of a tubular excretory system, equality of changes in the two clearances indicates that PAH extraction was not altered by specific tubular deficiency, even at the 28th day, although data from both clearances at day 28 were highly variable.

(3) UVNa. Means of this function were definitely increased by irradiation throughout the period of observation and, while highly variable, differences between experimental (post-irradiation) periods and controls are significant, indicating diminished capacity to retain Na on the part of the irradiated kidney.

(4) UVK. This, like the capacity to retain Na, was seriously impaired on the 3rd and 7th post-operative days. By the 14th day it had returned approximately to normal and, on the 28th day had fallen to subnormal levels.

(5) V. Urine volume from the irradiated side was significantly increased from the 3rd through the last post-irradiation day, and was highly variable -- depending largely on levels of CCr and CO_{Hg} by the 28th day.

(6) $T_{H_2O}^{C}$. This function was unchanged on the 3rd post-irradiation day; hence the increase in V observed at this time must have reflected osmotic diuresis consequent solely on decreased electrolyte (Na and K) reabsorption and not a defect in free water reabsorption. However, by the 7th day, the function of water reabsorption had seriously deteriorated; at day 14 it reached about 10 per cent of the rate in the untouched kidney and, by days 21 and 28 was negative, i.e., solute-free water was being excreted rather than retained on the irradiated side. The initial decrement, (day 7) in this function and the later decrement (day 21) were associated with large increases in means of V, the latter apparently reflecting the electrolyte-rich and free water loads under current excretion.

II. Effects of Drugs Affecting Electrolyte and Water Outputs by Enzymatic Actions.

The experiments described below were based on the following considerations. (a) Na^{+} and K^{+} exists in equilibrium with H^{+} in tubule cells and tubular fluid, this equilibrium being affected in the direction of "fixed base" (Na^{+} and K^{+}) conservation by tubular secretion of ammonia (NH_3), formed from glutamine principally, and whose formation is enhanced by infusion of glutamine in dogs (Van Slyke, Phillips, Archibald, Hamilton, Futcher and Hiller, J. Biol. Chem. 150:481, 1943) and, by addition of ammonium NH_4^{+}, which is available for exchange with Na^{+} and K^{+}; (b) that the supply of K^{+}ions for NH_4^{+} formation or exchange with Na^{+} or K^{+} depends on secretion of H^{+} by the action of intra-cellular carbonic anhydrase and (c) that, under conditions of osmotic diuresis, as in the present experiments, the urine formed is a "distal" fluid representative of tubular fluid after assembly of final electrolyte components of urine.

(1) Glutaminase was considered that irradiation might establish a defect in renal glutaminases such that NH_3 secretion, under control conditions or as augmented by infusion of glutamine, might be defective, with resultant loss of Na^{+} and K^{+} consequent on lack of NH_4 for exchange.

The first approach was measurement, at rest, of urinary titratable acidity and ammonia. This was not practical, since the bladder pouches, even when freshly cleansed, seemed nearly always to contain urea-splitting bacteria, leading to artefactually high contents of ammonia, while the pouches lost CO_2 to the air. Several direct measurements of ammonia were done (Conway method): some of these indicated the artefactually high contents noted, while other yielded apparently equal outputs as between the 2 sides. These data are not listed.

Next, 2 dogs were tested at 3 and 7 days after irradiation, as to the effect of glutamine. This was given intravenously in a rest period in a priming dose of 1.6 gm. and sustained by infusion at 3 mg. per min. during urine collection. Results are shown in Table IIA. Anticipated results, if glutaminase were seriously affected, were relatively greater depression of Na and K outputs on the untouched than on the irradiated side. This was observed, with regard to Na, in 1 dog, (SPO at days 3 and 7, but without corresponding changes in K and

with no changes in urine Volume (V). The experiments were considered suggestive, but not conclusive. They were not extended because experiments detailed below suggest that the defect observed in the 1 dog may have been attributable to deficit in H^+ secretion rather than secretion of NH_3.

(2) Carbonic Anhydrase. Renal carbonic anhydrase is almost completely inhibited in dogs by a single intravenous dose of about 10 mg. dichlorphenamide per kg. (personal communication, Dr. Karl Beyer, Merck, Sharpe and Dohme, who graciously furnished the drug for these tests). The drug was given in a slow, single intravenous dose during a rest-period after control collections and followed 5-10 minutes later, by collection of urine over 3 - 10 minute periods. Data are summarized in Table IIB. Increments in output (microequivalents per min.) of Na^+ and K^+ were always (from the 3rd through the 28th post-irradiation day) greater on the untouched than on the irradiated side, with roughly corrsponding greater increments in urine flow (V).

(3) Mercurials. The next class of agents tested was the mercurial diuretics, specifically, mercuhydrin, given in a single, slow intravenous dose of 1 mg. per kg. as was dichlorphenamide. Effects are summarized in Table IIC. These show that the untouched kidney in 1 of 2 experiments on the 3rd post-irradiation day, and in 4 experiments done on days 21 and 28 had greater Na^+ outputs than the irradiated kidney and that a like sequence prevailed as concerns K^+; 3 experiments showed little difference in water output between the 2 kidneys and 3 a lower output on the irradiated sides.

(4) Quinone. Correspondence with Dr. William Brodsky (University of Louisville) suggested consultation by letter with Dr. R. Kessler of Cornell (New York) Medical College, who graciously provided samples of a quinone which relatively specifically interfers with electron transport and H^+ secretion by kidneys, with resultant increases in Na, K and water output after its intra-renal arterial injection. This material was dissolved in ethanol, made up to 10 ml. volume in 1% NaCl solution and injected by Dr. Nolan, just above orifices of renal arteries in 2 dogs (table IID) in doses of 54 mg. The first experiment (DA, day 7) showed that the anticipated effects occur and are greater on the untouched than on the irradiated side. The second (BS, day 28) shows no effect on the untouched, but severe depression of Na, and moderate decreases in K and water outputs on the irradiated side. Both dogs died shortly after the injections, apparently from toxic effect of the drug. It is assumed that the untoward responses in the second experiment reflect beginning vascular collapse, so that ratios of renal response (L/R) reflect actual stimulation of Na, K and water outputs on the untouched (R) relative to the irradiated (L) side.

III. Actions of Angiotensin on Irradiated Kidneys. Wilson (proc. Prague Symposium on Essential Hypertension, State Medical Publishing House, Prague, 1960), with Asscher and Anson (Lancet:i:580, 1961) described increased sensitivity of mesenteric arteries of rats to arteriolar necrosis from even mild renal hypertension, after irradiation thereof. Recently, Asscher and Anson (Lancet, ii:1343, 1962) have shown that the same applies to cerebro-spinal vessels of rats. The irradiated vessels, prior to exposure to increased arterial pressure, showed no demonstrable histological changes. Hence, it seemed likely that the irradiated vascular muscle had somehow become abnormally sensitive to the destructive effects of increased lateral pressure. On the other hand, the possibility that increased sensitivity to the presumed pressor agent of renal hypertension, angiotensin, with resultant severe and, in some way, destructive vasoconstriction is not excluded. Further, angiotensin

has, under suitable conditions, a diuretic-saluretic effect which we (del Greco, Corcoran and Page, A. J. Physiology, 191:525, 1957) have previously suggested might be related to a change in the distribution of renal blood flow. Accordingly, effects of angiotensin were studied in the dogs described above.

As in other tests, observations were made (C_{PAH}, C_{Cr}, C_{Osm} and V (ml. per min.) and output of Na (micro-equivalents per minute) during control periods and during infusion of angiotensin-containing test solutions. Angiotensin doses delivered were usually 0.1 micrograms per min.: exceptions were dogs DA and ES (day 3, 0.15 micrograms per minute, DA (day 7, 0.15) dogs GG and SA (day 21, respectively 0.17 and 0.15) and dogs GG, SA and ES (day 28, respectively 0.12, 0.15 and 0.15). These dosages yielded only small and usually transient pressor effects.

Data are summarized in Table III, and are considered in relation to the separate functions measured, and, as before, as ratios of test/control period means and by comparison of these ratios with ratios of responses in left (irradiated) and right (untouched) kidney.

(a) C_{PAH}. On the right experiments at day 3 showed no mean renal vasoconstriction (11 per cent decrease in C_{PAH} at day 7, an apparent larger mean decrement in the 3 experiments at day 14, and mean decrements again (-13, -18 per cent) respectively at 21 days and 28. In contrast, the left kidney showed changes parallel to the right at day 3 (mean L/R 0.99), a somewhat greater decrease than the right (-18 per cent, L/R, 0.90) at day 7, and at day 14 (L/R, 0.91) a still greater decrease (mean L/R, 0.83)/and a large difference (-41 percent, L/R, 0.72) at day 28.
on day 21,

(b)C_{Cr}. This function was possibly somewhat increased in most experiments; at days 3 and 7 and equally on the two sides (respectively L/R means 0.99, 0.98), was bilaterally, equally somewhat depressed on day 14 (L/R mean 1.00) and was depressed on the irradiated as compared with untouched sides on days 21 and 28 (respective L/R means 0.89 and 0.89).

(c) CO_{sm}. Changes in this function tended to parallel those of C_{Cr} through day 14. Thereafter, presumably because of absolute decreases in glomerular filtration and of UV_{Na} (see below) on the irradiated side, mean CO_{sm} was severely depressed by angiotensin on the irradiated side (day 21, L/R 0.65, day 28, 0.66).

(d) V. Urine Volume from the right kidney increased 10 per cent or more in 18 but did not decrease by this proportion in any 23 angiotensin infusions. Changes in V on the left side paralleled those of the right on day 3 (L/R mean 1.03), but showed progressively diminishing diuretic responses to angiotensin on suceeding test days (means L/R=0.90, day 7; 0.81, day 14, 0.59, day 21 and 0.57, day 28).

(e) UV_{Na}. On day 3, 3 dogs (FR, SP and DA) showed sharp decreases in this function on the irradiated side and 2 (ZO and ES) some relative natriuresis on the irradiated side, so than mean L/R ratio was nearly unchanged (0.96). However, on day 7, only 1 dog (ZO) showed a left natriuretic response equal to the normal side, while in 3 the response was less, so that mean L/R response ratio was reduced to 0.84. On day 15, dog GG (not previously tested in this manner) showed a natriuretic response to angiotensin on both sides of about equal degree (L/R=1.14), but in 2 dogs the response was less, so that mean L/R response ratio was 0.88. By day 21, when GFR on the irradiated side has usually decreased below control levels, natriuresis was very defective during angiotensin infusion on the irradiated side (mean L/R response ratio 0.51, and the defect was more intense on day 28 (L/R=0.44 mean).

COMMENT.

I. Effects of Irradiation of Kidney Functions.

The relative levels of two functions, C_{PAH} and C_{Cr}, respectively taken as indicative of rates of renal blood flow and glomerular filtration, were significantly increased during the first weeks after the course of irradiation, this increased persisting, but diminishing somewhat, at the end of the second week. Those effects are interpreted as reflecting renal hyperemia, possibly consequent on an early "inflammatory" response, liberation from cells of local vasodilators i.e., mechanisms similar to those that provoke hyperemia in burns.

These functions diminish to control levels at the third week and fall below these levels - albeit irregularly, - at the fourth. This sequence is interpreted as the result of beginning tissues destruction and early interstitial fibrosis. Histologic data that might confirm these suppositions are not yet available.

The largest early changes in functions occurred in output of Na and K. These were doubled at days 3 and 7, diminished towards control levels as concerns K, but were still doubled as concerns Na at day 14, and with persistent natriuresis thereafter, inspite of decreasing filtration rate.

Possible mechanisms of this "salt loosing" state noted above, are considered below in more detail. However, at this point, it seems important to emphasize that the "water-loosing" defect does not appear until day 7 after the course of irradiation and that it progresses thereafter from a state (days 7 and 14) in which net conservation of free water ($T_m^c H_2O$) diminishes, to one in which there is net loss (actual clearance) of free water, i.e., a progressive defect in water reabsorption. To a degree these changes in water reabsorption are paralled by changes in V (rate of urine flow). However, it should be noted that the first increment in V (day 3) is apparently the result of osmotic diuresis, dependent on the primary failure in Na and K reabsorption, since it is not associated, at this time with a change in water reabsorptive capacity. Thereafter, changes in V reflect the combination of 2 factors, i.e., osmotic diuresis and failure of free water reabsorption.

The above describes and attempts to interpret the sequence of events. Among these, the most significant seems to be the failure --evident at day 3) in Na and K reabsorption and the next failure of reabsorption of free water, which begins between day 3 and day 7 and progresses through day 28.

A possible interpretation of this sequence could be based on the "counter-current" concept of electrolyte-water reabsorption in renal tubules, and on the fact that blood flow to renal medulla is relatively low (roughly 5 per cent of total flow). Thus, if, during osmotic diuresis, as in these experiments or, possibly even in the resting state, the irradiated kidney is constantly subject to depletion of electrolytes, ultimately possibly by transfer of part of these from medullary interstitial fluid, this transfer should ultimately deplete medullary electrolytes and change the osmotic milieu thereof from its normally hypertonic state - upon which reabsorption of free water largely depends - to one of diminishing osmolarity. While, this view may explain the sequence through day 14, - - on the possibly inadequate assumption that medullary blood flow somehow does not keep up with medullary depletion of electrolytes - it does not explain the sequence thereafter, in which there occurs net loss of free water.

Hence, it seems likely that the major early functional effects of renal irradiation are to impair first mechanisms of Na and K reabsorption or conservation and secondly mechnisms of water transport. Electrolytes at least are believed to consume much of the energy the kidneys use. The latter transport system is usually visualized as passive to a degree, - - assuming medullar hyperosmolality, and dependent on permeability of

tubular cells (distal and convoluted). It is normally, regulated by variations in permeability consequent on changes in secretion of anti-diurectic hormone (AHD). Under the conditions (anesthesia, hydropenia, osmotic diuresis) of these experiments, secretion of ADH should be close to a physiologic maximum. Hence it seems that the tubular mechanisms, sometimes visualized as "pores" that respond to ADH and regulate cell permeability, have become irresponsive to this physiologic stimulus as a result of irradiation.

II. Nature of Defect in Electrolyte Transport. As noted above, Na^+, K^+ and H^+ exist in equilibria, determined by mechanisms of Na^+ transfer and H^+ secretion in renal cells and tubular fluid. Accordingly, experiments were done with drugs that have ultimately similar effects, in that they induce increased Na^+ and K^+ output, usually associated with diuresis, but which act on difference cellular mechanisms. The aim was by comparing relative effects on normal and irradiated kidneys, to determine if such system was disproportionately or predominantly affected by irradiation.

The experiments with glutamine were, at most, only suggestive that there might be a diminished availability of H^+; they seemed (as did ammonia determinations not listed above) to exclude ammonia-secretion by glutaminase activity as a primary mechanism in the electrolyte loss. The experiments with dichlorphenamide, are consistent with the views either (a) that the carbonic anyhydrase system and its provision of H^+ is predominantly affected by irradiation or (b) that cellular systems for K^+ transfer are impaired, even though the enzyme mechanism in the cell may be intact. The experiments with mercuhydrin show net effects somewhat similar to those with dichlorphenamide, with the exception that urine volume was not as much increased during its action in either normal or irradiated kidneys, in most experiments. Since mercuhydrin apparently acts on systems other than carbonic anyhydrase in suppressing electrolyte excretion, these experiments would be consistent with the view that irradiation impairs basic mechanisms of electrolyte, presumably H^+ transfer and notthe view that acts primarily or predominantly on the carbonic anyhydrase system.

Parenthetically, it should be noted that 2 experiments using as "natriuretics" (chlorothiazide and hydrochlorothiazide; as in the experiments with dichlorphenamide and mercuhydrin, quantitative differences were present, with diminished responses from the irradiated kidneys. These data are not listed, since they seem merely confirmatory of the above conclusion and are too few in number for separate consideration.

Lastly, the quinone compound used in the 2 experiments listed at the end of Table II is believed specifically to interfere with a basic mechanism (Coenzyme Q) of proton transfer and H^+ transport in tubule cells (Weinstein S., Kessler, R. H. Fed. Proc. 21:431:1962). Assuming this to be the case, the 2 experiments suggest that this may be the ultimate mechanism affected by irradiation. Responses of the irradiated kidney in electrolyte and water outputs were grossly defective as compared with that of the normal.

III. Actions of Angiotensin. The effects of irradiation on renal actions of angiotensin seem to reflect two sites of action, viz., (a) the renal arterial vasculature, which becomes more sensitive to the vasoconstrictor effect of the agent and (b) renal tubules, which become less sensitive to its diuretic-natriuretic action.

The mechanism by which irradiation might sensitize to vasoconstriction is not at all clear except as it may be related to a change in H^+ transfer, such as is assumed above to underlie the effect of irradiation on tubular electrolyte transport. There is a body of evidence that vasocosntriction in arteries and arteriolesm is strongly dependent on ion shifts with initial Na^+ ingress, concurrent transient K^+ loss, and, presumably,

redistribution of H+ (Friedman, S. M. et al. Sodium Ion and Smooth Muscle Contraction. Hypertension, Vol. VIII. (Proc. Council High Blood Press. Res., Amer. Heart Assoc., New York, 1959). An economy of hypothesis is achieved if the tubular and renal vascular effects of irradiated kidneys can be linked as reflecting a common change in basic cellular energetics.

The relative unresponsiveness of the irradiated kidneys to the natriuretic and diurectic effects of angiotensin is qualitatively and quantitatively similar to the relative unresponsiveness of tubular mechanism to drugs listed above that depress Na^+, K^+ and (as is assumed) ultimately H^+ transport. Presumably, this reflects a common mechanism of irradiation effect. It is of interest because, as noted above, we had previously considered it likely that the saluretic-diuretic effects of angiotensin might reflect some change in the distribution of renal blood (e.g. increased medullary blood flow) rather than a direct tubular action of this agent which, as far as is generally recognized has primarily a myotropic action on smooth and cardiac muscle. The present experiments indicate that the action of angiotensin on urine volume and sodium content are <u>tubular</u> rather than <u>vascular</u> and reflect an action of the hypopeptide on some mechanism of Na^+ transfer possibly in both tubular vasodilar cells.

<u>IV. Direction of Further Experiments.</u> These are suggestions only. The general objective of this study, viz., definition of a basic cellular change occuring early after irradiation, using the kidney as a test system because its two fluids, plasma and urine, were simultaneously available for analysis, has been achieved. However, no more than a broad definition of the ultimate mechanism affected has been realized. There seem, in fact to be possibly two. One concerns H^+ transport and availability; apparently this is early impaired by severe irradiation and underlies the Na^+ and K^+ loosing-state induced in the irradiated kidney, its relative unresponsiveness to agents stimulating Na^+ loss, including angiotensin (which now seems to have a direct action on renal tubular cells), and, as suggested above, may account for the enhanced responsiveness to angiotensin of the irradiated renal arterial system. The second effect in temporal sequence is impairment of permeability of cells to water.

While it may be possible, as by micropuncture techniques, to demonstrate and substantiate this concept in intact kidneys, it would seem more profitable to explore the concept in simple systems, capable of energy-dependent electrolyte and water transport. One such, the turtle bladder has been extensively studied by Brodsky. It would also be of interest to irradiate large vessels such as the aortas of rats or rabbits and then, by testing responsiveness of aortic strips and measuring energetics and electrolyte fluxes in such isolated systems to determine the nature of the change noted in the renal vascular response to angiotensin. The experiments with reference to irradiation as such, a study of its effects on membrane that transport electrolytes and water.

In brief, while the kidney lends itself to the demonstration of basic changes in electrolyte and water transport induced by severe irradiation, the intact kidney is far too complex a structure to enable analysis of this change in ultimate detail.

SUMMARY AND CONCLUSIONS.

1. Severe irradiation (2400-3000 r in 3 divided doses) of dog kidneys induced, even at the 3rd post-irradiation day, a state of Na^+ and K^+ loss as compared to the normal kidney.

2. This is followed, after some days, by impairment of water-reabsorbing capacity.

3. The electrolyte-loosing state is associated with relative impairment of response to a variety of agents that, by different mechanisms, suppress electrolyte reabsorption. The experiments indicate that angiotensin seems to be one of these, i.e., that angiotensin has a renal tubular as well as vasoconstrictor action.

4. The vasculature of the irradiated kidneys develops increased vasoconstrictor responsiveness to angiotensin.

5. The ultimate mechanism of the changes observed is not clear. However, the fact that two very difference tissues, viz., vascular renal smooth muscle and renal tubular electrolyte transport, are concurrently affected, together with 2 experiments with a drug that is believed to impair primary energy transfer systems of cells, suggest that the basic defect induced by irradiation soon after its administration lies at this basic cellular level of proton transfer and H^+ transport across cell membranes.

6. Suggestions are made that studies be extended to discrete preparations of isolated tissues, including turtle bladder and rodent aorta, with the aim of defining mechanisms in simpler systems than the intact kidney.

SECONDARY OBJECTIVE

As noted above, equipment was provided for radioisotope renography and there were intervals between experiments relating to the primary objective which were advantageously filled by evaluation of this technic.

BACKGROUND AND COURSE OF STUDY.

Introduced in 1956, the radioisotope remogram had been used in several centers with the aim of delineating unilateral renal disease by comparisonof patterns traced over each kidney site. The technique was greatly improved in 1958 by the introduction of I^{131}-hippuran, which, unlike I^{131}-Diodrast, does not tend to concentrate in liver and distort the tracing from the right kidney. While claims had been made as to the interpretation of the normal triphasic curve in terms of an initial "vascular peak" - apparently representing renal blood flow, a second "tubular accumulation phase" -- loosely considered a matter of tubular uptake and storage of isotope and a third, "excretory" phase of downslope, these interpretations had been applied clinically, arbitrarily and empirically and without experimental validation.

Accordingly, an experimental study of components of the renogram was undertaken with Dr. Klapproth. A note on this (Corcoran, A. C. Med. Clin. N. A., Vol. 45 #2, Mar.,1961) The material was presented by Dr. Klapproth at the Annual Meeting of the Amer. Urol. Soc. and later published (Klapproth, Hirakawas and Corcoran, J. Urol. Vo. 87, No. 1. Jan. 1962). The paper describes components of the renogram, the effect thereon of physiological changes, such as segmental and main renal artery ligation or partial stenosis, infusion of pharmacologic agents, such as norepinephrine and angiotensin etc,, and of administration of large doses of PAH (aimed at suppressing tubular transfer of the isotope carrier). The net conclusion was that the renogram, as currently depicted, is a pattern reflecting multiple, kinetic changes in renal function and that no segment can be simply interpreted in terms of a discrete renal function. Thus, the "vascular spike" was shown to be about 50 per cent tubular accumulation and, under standard conditions with the kidney in situ, 20 per cent or more "background" i.e., radioactivity in large vessels other than renal. The phases of "tubular accumulation" and "excretion" are simply representative of times in the sequence when blood isotope level has fallen sharply below its initial concentration and, in the case of the former, accumulation and transfer of isotope into tubular fluid is proceeding more rapidly than loss of this fluid from the kidney, while during the "excretory" phase, the rate of excretion exceeds that of uptake. This study provided a firm, physiologic basis for continuing studies described below.

As the problem was visualized at that time, it seemed to us that (a) the renogram, for clinical purposes, was entirely too empirical in its interpretation (b) had no connotation numerically expressive of relative or absolute level of overall renal function and (c) that, in enthusiasm raised by the misinterpretation of the initial phase as a "vascular spike" and current interest in renal arterial occlusive disease, too much attention was being paid the possibilities and defects of this technique in this particular form of renal disease and too little in its more general application as a screening test of renal function.

The renogram is usually described as having 3 major components. These are (a) a steep rise form base line point 0, beginning within a minute after intra-venous injection of isotope-carrier (I^{131}-hippurate, Hipputope^{R}, in these experiments, graciously furnished by Dr. Paul Numerof, E. R. Squibb and Sons, New Yrok, N.Y.) which comes to a "shoulder"(Characterized as point A) and then rises more slowly for

2 - 3 minutes to a peak, point B, from which it falls at first rapidly and then more slowly to some terminal point (chosen at 15 minutes after injection in pur experiments), point C, where the record terminates. The slope O-A is commonly called a "vascular spike" with the implication that its represents inflow of blood into the kidney; our experiments in dogs showed that this is true in part, but that a substantial part of this slope represents "background" of isotope in great vessels and muscles and another fraction storage of isotope in tubular cells and fluid at a faster rate that it is being excreted; at about 2 minutes, excretion exceeds uptake from blood and the curve falls off from point B, rapidly atfirst as the higher concentration is cleared and more slowly as less remains in the kidney and blood level falls precipitously with distribution of the carrier and isotope in a large fraction of body fluid.

Basically, the function of a kidney is to extract and then to excrete. Total function can be visualized as the sum of these functions. The rise perpendicularly from point A to B can be visualized as reflecting extraction: the perpendicular from B-C represents excretion during the time chosen (15 min.) for recording. Neither is an absolute indication of its specific function. As noted, it would be desirable to chose a point A, probably mid-way along the slope O-A in normal subjects, to indicate beginning extraction and eliminate a large part of background. This is impractical, since, with diminished function of extraction, it would be impossible to place this hypothetical point. Accordingly, the distance in mm. $(B-A)^2$ was taken as a measure of extraction and mm. $(B-C)^2$ of excretion and the sum of these divided by the perpendicular $(B-O)^2$ to yield an index which, for convenience, was multiplied by 100 to yield a Renogram Index, indicative of separate total function of each kidney.

Mean normal R. I. fortuitously corresponds approximately to concurrent excretion (percentile) of injected isotope by the 2 kidneys in 20 minutes. Thus, measurement of excretion rate in 20 minutes yields an indication of total and measurement of bilateral R. I. indicates contributions thereto of each kidney. Comparisons of R. I. values in subjects with symmetrical renal disease indicated that measurement which fell within 15% of each other were within 1° standard deviation of their mean, and of concurrent excretion rate down to excretion rates of approximately 50 per cent of normal, i.e., 30 per cent of injected isotope.

The procedure, its theory and practice and results in patients are described in detail in a paper now in press in J. Lab. Clin. Med. (1953) and need not be elaborated here, except to add that this constitutes a widely applicable, sensitive test of renal function which supplements information derived from other procedures and, in some patients, may point to the desirability of further, more complicated and uncomfortable (measurement of separate renal functions by ureteral catheterization, renal angiography) procedures. However, like most procedures, it is not fully diagnostic of renal arterial stenotic lesions and our experience shows that this diagnosis is only finally accomplished by renal angiography. Reprints, as available will be furnished, as has been a copy of the original MS.

The technique has been demonstrated in an exhibit shown at the IV International Cardiological Congress, Mexico and again at the Annual Meeting of the American Heart Association, (Cleveland) respectively in October and November, 1962. It will ne shown again at the Cleveland(Annual) Meeting of Ohio State Medical Association, May 12-14, 1963.

The experiments have, since the termination of the contract, been extended to computation of renal blood flow from renogram records, using analogue computers and the cooperation of the Computer Division, Case Institute of Technology. An Abstract on this application was published in Circulation, Nov. 1962 and in J. Lab. Clin. Med. Nov. 1963. These experiments are still in progress.

TABLE I.

MEANS OF SEQUENTIAL EFFECTS OF IRRADIATION ON RENAL FUNCTIONS.

	Numbers of Animals	CPAH	CCr	V.	$Tm^{c}H_2O$	UVNa	UVK
		ml. per min.				microEq/min.	
Pre-irradiation	13	98±6	98±6	99±9	99± 11	93±28	99±12
Post-irradiation days							
3	9	118±12*	123±16*	149±10*	106±23	202±93*	254±150
7	6	120±15*	126±17*	189±60*	53±33*	203±101*	194±34*
14	5	113±4*	108±4*	156±47	9±107*	200±173*	128±40
21	5	106±25	104±17	285±81	-68±63*	476±227*	96±36
28	5	69±62	58	varied	-90±78*	285±274*	52±41*

Legend. Effects are expressed by assuming function of untouched kidney is 100 and expressing function of irradiated kidney as percentage of this in 3 first control periods of each experiment; standard deviation is usually indicated and significant differences noted by an asterisk (P greater than 0.01) .x as calculated for small groups.

TABLE II

EFFECTS OF AGENTS AFFECTING ELECTROLYTE AND WATER OUTPUTS IN NORMAL AND IRRADIATED KIDNEYS.

(ratios means of test/control periods and of right untouched to left irradiated kidneys).

Section	Drug	Dog	Day	Na output			K output			Vol. output		
				R	L	R/1	R	L	R/L	R	L	R/L
A	Glutamine	SP	3	0.71	1.20	1.69	1.26	1.33	1.06	0.88	0.93	1.06
		SA	3	0.86	0.83	0.97	0.82	0.77	0.94	0.96	0.90	0.94
		SP	7	0.69	0.94	1.36	0.92	1.01	1.10	0.86	0.88	1.02
		SA	7	2.00	1.65	0.83	1.30	1.35	1.09	1.29	1.21	0.94
B	Dichlorph.	FR	3	3.09	2.18	0.71	5.05	2.91	0.58	1.90	1.56	0.82
		ZO	3	9.38	7.45	0.90	4.25	3.80	0.89	1.88	1.92	1.02
		ZO	7	14.80	7.03	0.48	6.04	3.39	0.59	2.83	2.27	0.80
		GG	14	1.74	1.37	0.79	2.50	1.91	0.76	1.37	1.13	0.82
		ZO	14	2.58	1.65	0.64	2.08	1.76	0.85	1.28	1.08	0.78
		GG	21	2.22	2.00	0.90	4.23	3.20	0.76	1.45	1.14	0.79
		GG	28	5.14	1.67	0.30	2.82	2.18	0.77	1.59	1.03	0.65
		ZO	28	13.68	1.47	0.11	1.95	1.22	0.63	1.71	1.03	0.60
C	Mercuhyr.	DA	3	4.45	2.38	0.53	2.80	1.63	0.58	1.87	1.81	0.97
		ES	3	1.49	1.66	1.01	1.12	1.05	0.94	1.55	1.73	1.11
		SP	21	2.45	1.36	0.55	1.07	0.58	0.54	1.56	1.03	0.66
		SA	21	4.43	2.63	0.59	1.68	1.16	0.69	3.04	1.92	0.63
		SP	28	3.35	1.37	0.41	1.03	0.86	0.83	1.06	1.34	1.26
		SA	28	2.00	0.90	0.45	1.47	0.87	0.59	1.48	0.95	0.64
D	Quinone	DA	7	7.79	5.06	0.65	5.28	3.21	0.61	4.91	2.98	0.61
		ES	28	1.00	0.09	0.09	1.08	0.62	0.57	1.02	0.67	0.66

Legend. Effects in test/control periods of agents affecting Na, K and electrolyte output in normal, untouched (R) and irradiated (L) kidneys of designated animals at indicated days after X-irradiation and relative effects on irradiated/control kidney (R/L).

TABLE III.

RATIOS OF RESPONSIVENESS OF RENAL FUNCTIONS TO ANGIOTENSIN INFUSIONS AFTER X-IRRADIATION.

(means of test periods ′means of control periods of urine collection)

Days	Dog	CPAH			CCr			COsm			V			UV Na		
		R	L	L/R	R	L	L/RL	R	L	L/R	R	L	L/R	R	L	L/R
3	FR	0.97	1.14	1.17	0.94	1.07	1.14	1.25	1.34	1.07	1.13	1.25	1.11	2.02	1.42	0.71
"	ZO	1.53	1.53	1.00	1.05	1.08	1.03	1.37	1.46	1.07	1.59	1.57	0.99	1.85	2.21	1.20
"	SP	0.99	0.87	0.88	1.18	1.19	0.99	1.82	1.88	1.03	2.37	2.35	0.99	3.66	3.24	0.89
"	DA	1.11	0.97	0.89	1.25	1.02	0.81	1.59	1.30	0.82	2.26	2.06	0.91	5.20	2.24	0.43
"	SA	0.91	0.92	1.01	1.00	0.98	1.02	1.10	1.12	1.02	1.29	1.41	1.09	1.48	1.42	0.95
"	ES	0.95	0.93	0.98	1.11	1.09	0.99	1.37	1.38	1.01	1.51	1.65	1.09	1.23	1.99	0.89
"	Means	1.08	1.06	0.99	1.09	1.07	0.99	1.37	1.38	1.01	1.68	1.72	1.03	2.57	1.99	0.96
7	ZO	0.85	0.66	0.77	1.01	1.05	1.04	1.24	1.30	1.05	1.28	1.31	1.02	1.61	1.80	1.12
"	SP	0.73	0.72	1.01	1.04	1.19	1.15	1.52	1.41	0.93	1.77	1.43	0.81	2.89	1.78	0.62
"	Da	1.19	1.11	0.85	1.39	1.26	0.90	1.88	1.73	0.92	2.68	2.23	0.82	4.15	3.64	0.88
"	SA	0.79	0.77	0.97	1.05	0.88	0.84	1.14	1.03	0.90	1.09	1.02	0.94	1.55	1.14	0.74
	Means	0.89	0.82	0.90	1.12	1.10	0.98	1.40	1.37	0.98	1.70	1.50	0.90	2.55	2.09	0.84
14	GG	0.74	0.67	0.90	0.90	0.88	0.98	1.18	1.34	1.14	1.72	1.47	0.82	1.54	1.61	1.14
"	ZO	0.81	0.86	1.05	0.85	0.98	1.15	0.84	0.97	1.15	1.03	0.92	0.89	1.09	0.95	0.87
"	ES	0.71	0.60	0.78	1.07	0.95	0.88	1.32	1.05	0.80	1.56	1.13	0.72	2.30	1.45	0.63
	Means	0.75	0.71	0.91	0.94	0.94	1.00	1.11	1.12	1.01	1.44	1.17	0.81	1.64	1.67	0.88
21	GG	0.72	0.66	0.91	1.08	0.89	0.82	1.44	0.85	0.59	1.78	0.91	0.51	2.13	1.20	0.57
"	ZO	0.97	0.84	0.87	1.01	0.97	0.96	1.16	0.44	0.38	1.26	0.97	0.95	2.31	1.05	0.45
"	SP	0.90	0.75	0.83	1.11	0.98	0.88	2.00	1.73	0.87	2.84	1.71	0.60	8.95	3.70	0.41
"	SA	1.06	0.77	0.72	1.16	1.08	0.92	2.09	1.22	0.58	2.26	1.11	0.49	3.43	1.50	0.44
"	ES	0.60	0.36	0.60	0.94	0.82	0.87	1.02	0.84	0.82	0.97	0.51	0.52	1.12	0.77	0.69
	Means	0.86	0.71	0.83	1.06	0.95	0.89	1.54	1.02	0.65	1.84	1.09	0.59	3.59	1.64	0.51
28	GG	0.75	0.64	0.85	1.00	0.84	0.84	1.14	0.82	0.72	1.25	0.87	0.69	1.50	1.08	0.72
"	ZO	0.91	0.84	0.92	1.07	0.85	0.79	1.15	0.78	0.68	0.93	0.73	0.79	1.50	0.68	0.45
"	SP	0.81	0.50	0.62	1.01	0.98	0.99	1.24	1.03	0.83	1.55	1.00	0.64	3.28	1.21	0.37
"	SA	1.05	0.62	0.59	1.12	1.10	0.98	1.76	0.68	0.39	1.72	0.57	0.34	2.55	0.55	0.22
"	ES	0.60	0.36	0.60	0.75	0.67	0.88	0.95	0.66	0.69	0.97	0.51	0.52	0.92	0.41	0.44
	Means	0.82	0.59	0.72	0.99	0.89	0.	1.25	0.79	0.66	1.28	0.73	0.57	1.95	0.79	0.44